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Dark Respiration Protects Photosynthesis Against Photoinhibition in Mesophyll Protoplasts of Pea (*Pisum sativum*)[1]

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ABSTRACT

The optimal light intensity required for photosynthesis by mesophyll protoplasts of pea (*Pisum sativum*) is about 1250 microeinsteins per square meter per second. On exposure to supra-optimal light intensity (2500 microeinsteins per square meter per second) for 10 min, the protoplasts lost 30 to 40% of their photosynthetic capacity. Illumination with normal light intensity (1250 microeinsteins per square meter per second) for 10 min enhanced the rate of dark respiration in protoplasts. On the other hand, when protoplasts were exposed to photoinhibitory light, their dark respiration also was markedly reduced along with photosynthesis. The extent of photoinhibition was increased when protoplasts were incubated with even low concentrations of classic respiratory inhibitors: 1 micromolar antimycin A, 1 micromolar sodium azide, and 1 microgram per milliliter oligomycin. At these concentrations, the test inhibitors had very little or no effect directly on the process of photosynthetic oxygen evolution. The promotion of photoinhibition by inhibitors of oxidative electron transport (antimycin A, sodium azide) and phosphorylation (oligomycin) was much more pronounced than that by inhibitors of glycolysis and tricarboxylic acid cycle (sodium fluoride and sodium malonate, respectively). We suggest that the oxidative electron transport and phosphorylation in mitochondria play an important role in protecting the protoplasts against photoinhibition of photosynthesis. Our results also demonstrate that protoplasts offer an additional experimental system for studies on photoinhibition.

Photoinhibition is the phenomenon of a severe reduction in photosynthetic performance under supra-optimal light intensities, particularly in the absence of CO_2 and O_2 (10, 15, 22). Photoinhibition occurs *in vivo* when the photosystems absorb light in excess of their capacity of energy dissipation (10). The susceptibility to photoinhibition is usually enhanced under environmental stresses like drought, high temperature, and chilling/freezing, or under conditions of depleted intercellular CO_2 concentration (*e.g.* see ref. 8).

The nature of photoinhibition is similar irrespective of the causal factor (14). Among the components of photosynthesis, PSII reactions are the most sensitive to photoinhibition in green algae as well as in higher plants (14, 23). Q_B protein,[2] an important constituent of the photosynthetic electron transport chain, is believed to be the primary target of photoinhibition. This highly dynamic protein is synthesized as well as degraded continuously. Photoinhibition becomes apparent when the damage caused by light exceeds the extent of repair (16, 20).

The phenomenon of photoinhibition has so far been demonstrated in leaves, algal cells, chloroplasts, and thylakoid membranes (reviewed in refs. 10, 14, 15; see also refs. 17, 30). The present report describes the phenomenon of photoinhibition in another system, namely mesophyll protoplasts of pea. Protoplasts are useful tools to examine plant metabolism because they do not have any barrier against diffusion of O_2, pose no problem of recycling of gases within the intercellular spaces, and could further allow an evaluation of the externally added compounds. The interaction between photosynthesis and respiration within a leaf tissue has been a matter of intense debate (6, 7). However, recent reports establish that there is a rapid and strong interaction between respiration and photosynthesis in plant tissue. Respiratory metabolism is beneficial for photosynthesis in the plant cell, as shown in leaves and protoplasts (12, 13, 28). The rate of dark respiration in leaves is increased after prolonged illumination and is presumed to be one of the long-term effects of photosynthesis. This phenomenon, which was recently termed LEDR, is demonstrated in leaves (26) as well as in protoplasts (24), even during short periods.

The present article demonstrates that dark respiration could protect the protoplasts against photoinhibition of photosynthesis. Our results suggest that the oxidative electron transport and phosphorylation in mitochondria play a much more important role than the reactions of glycolysis or tricarboxylic acid cycle in protecting the protoplasts against photoinhibition.

MATERIALS AND METHODS

Plant Material

Plants of pea (*Pisum sativum* L. cv *Arkel*) were raised in plastic trays filled with soil and organic manure. The plants were grown outdoors under a natural photoperiod of approximately 12 h and average daily temperature of 30°C during the day and 20°C at night.

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[2] Abbreviations: Q_B protein, 32-kD herbicide binding protein of PSII that binds Q_B; LEDR, light enhanced dark respiration; PCR, photosynthetic carbon reduction.

Protoplast Isolation

The mesophyll protoplasts were isolated from first and second fully unfolded leaves of 8- to 10-d-old plants, as already described (4, 28), with a few modifications. The leaf pieces stripped of their abaxial (lower) epidermis were left for 30 min in a Petri dish containing preplasmolysis medium of 0.3 M sorbitol and 1 mM $CaCl_2$ in 10 mM Mes-KOH, pH 6.0. The strips were then subjected to digestion in a medium composed of 2% (w/v) Cellulase Onozuka R-10, 0.2% (w/v) Macerozyme R-10 (Yakult Honsha Co. Ltd., Nishinomiya, Japan), 0.25% (w/v) BSA, 10 mM sodium ascorbate, 0.4 M sorbitol, 1 mM $CaCl_2$, and 0.25 mM EDTA, in 10 mM Mes-KOH, pH 5.5. Digestion was done at 30°C for 30 min under an illumination of 50 μE m^{-2} s^{-1}.

After digestion, the medium was gently removed and a few milliliters of washing medium containing 0.4 M sorbitol and 1 mM $CaCl_2$ in 10 mM Mes-KOH, pH 6.0, were added. Tapping and swirling of the Petri dish released a large number of protoplasts into the medium. The crude protoplast suspension was filtered successively through nylon filters of pore size 300 and 60 μm and centrifuged at 50*g* for 5 min. The pellet was washed twice with the washing medium and once with suspension medium of 0.4 M sorbitol, 1 mM $CaCl_2$, and 0.5 mM $MgCl_2$ in 10 mM Hepes-KOH, pH 7.0. The protoplast pellet was finally suspended in the above medium to give 200 μg Chl mL^{-1} and kept on ice.

O_2 Uptake/Evolution

Respiratory O_2 uptake in the dark and photosynthetic O_2 evolution in the light by protoplasts were monitored at 30°C using a Clark type O_2 electrode (model DW2, Hansatech Ltd., King's Lynn, UK). The reaction medium of 1 mL contained 0.4 M sorbitol, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $NaHCO_3$ in 10 mM Hepes-KOH, pH 7.5, and protoplasts equivalent to 20 μg of Chl. Water at a constant temperature of 30°C was circulated through the outer jacket of the reaction chamber by using a refrigerated circulatory water-bath. Illumination (1250 μE m^{-2} s^{-1}) was provided by a 35 mm slide projector (Atul Electronics Corporation, India; lamp: Xenophot [halogen], 24 V/150 W).

Photoinhibitory Treatment

Protoplasts (in the suspension medium and containing 200 μg Chl mL^{-1}) were exposed to supra-optimal light intensity (2500 μE m^{-2} s^{-1}) provided by two tungsten bulbs (Philips, Calcutta, India: Comptalux, 225 V/75 W), one on either side of the thermo-jacketed preincubation chamber at 30°C. Additional water filters between the bulbs and preincubation chamber prevented heating. For comparison, protoplasts were exposed to normal light (1250 μE m^{-2} s^{-1}) or kept in darkness at 30°C. The protoplast suspension was gently stirred during incubation in photoinhibitory/normal light or darkness.

Preincubation with Respiratory Inhibitors

The respiratory inhibitors were included during preincubation at the concentrations indicated in the text. The preincubation was done in the chamber (described above), kept either in darkness or under photoinhibitory light (2500 μE m^{-2} s^{-1}). The incubation was for 10 min unless otherwise specified. An aliquot (100 μL) was taken out and examined for metabolic activities with reference to control samples. A minimum of a 10-fold dilution of the preincubated samples avoided the direct effect, if any, of the inhibitors during assays.

Peroxide Scavenging Enzymes

Catalase (EC 1.11.1.6) activity was assayed by following the disappearance of H_2O_2 at 240 nm, using an extinction coefficient of 25 mM^{-1} cm^{-1} for H_2O_2 (27). Peroxidase (EC 1.11.1.7) was assayed using guaiacol as the substrate. The formation of tetraguaiacol was measured at 470 nm using an extinction coefficient of 26.6 mM^{-1} cm^{-1} (29). Both the enzymes were monitored in a Shimadzu UV-VIS Spectrophotometer (model UV-160A).

Other Procedures

Chl was determined after extraction into 80% (v/v) acetone (1). The experiments were repeated on different days. The data represent the averages (±SE) of results from at least three measurements.

RESULTS

The optimal light intensity required for maximal rates of photosynthetic O_2 evolution by mesophyll protoplasts of pea was 1250 μE m^{-2} s^{-1}. The maximum rates of CO_2-dependent O_2 evolution at 30°C ranged from 116 to 170 μmol mg^{-1} Chl h^{-1}.

The pattern of photosynthesis by mesophyll protoplasts after exposure to photoinhibitory light was compared to those kept in either darkness or the optimal light intensity of 1250 μE m^{-2} s^{-1}. There was a slight decrease in the photosynthetic rate of protoplasts when kept in dark or normal light. On the other hand, when protoplasts were exposed to supra-optimal light of 2500 μE m^{-2} s^{-1}, their photosynthetic rates declined rapidly with time (Fig. 1). Protoplasts lost nearly 35% of their photosynthetic activity after 10 min and approximately 60% of the activity by 20 min, with respect to dark-incubated samples, demonstrating that photoinhibition of photosynthesis occurred when protoplasts were exposed to strong light.

The rate of respiration was slightly stimulated when protoplasts were allowed to photosynthesize under normal light of 1250 μE m^{-2} s^{-1} (Fig. 2), but their respiration was markedly reduced when exposed to photoinhibitory light. The protoplasts lost about 40% of their respiratory activity after exposure to photoinhibitory light.

The response of protoplasts to photoinhibitory light was examined in the presence of classic inhibitors known to suppress different components of respiration: antimycin A, sodium azide, oligomycin (oxidative electron transport/phosphorylation), sodium fluoride, and sodium malonate (glycolysis/tricarboxylic acid cycle). Figure 3 presents the results of a typical experiment done with antimycin A. There was a

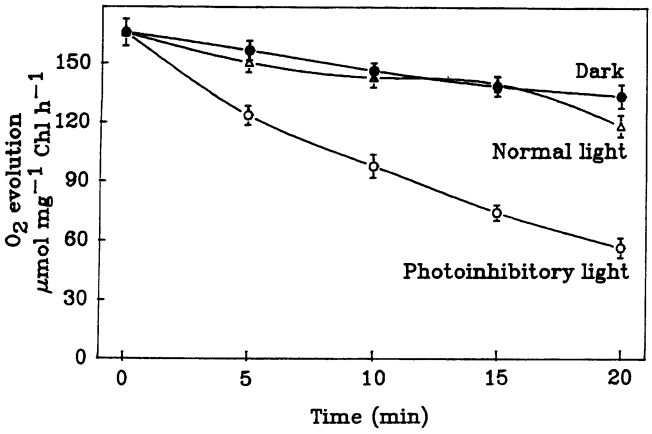

Figure 1. The rates of photosynthesis by mesophyll protoplasts at different intervals of time after incubation at 30°C in darkness or in light. When protoplasts were kept in normal light intensity (1250 μE m^{-2} s^{-1}), the loss was negligible (up to 15 min) or marginal (20 min). Photosynthetic rate declined steeply on exposure to supra-optimal or photoinhibitory light (2500 μE m^{-2} s^{-1}). The Chl concentration during preincubation was 200 μg Chl mL^{-1}.

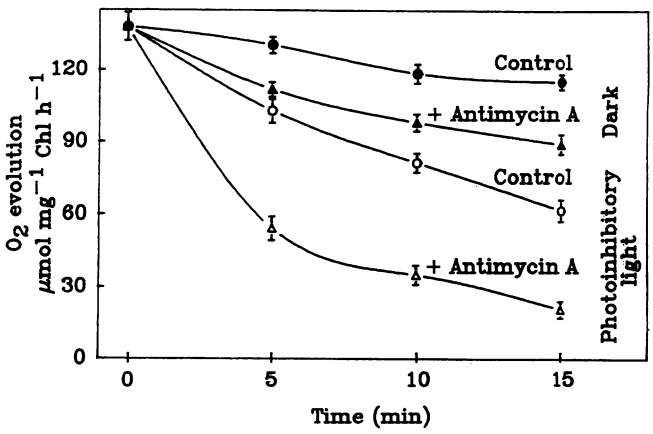

Figure 3. Effect of 1 μM antimycin A on the extent of photoinhibition of photosynthesis. Shown is the rate of photosynthesis at different intervals of time when protoplasts were preincubated without (control) or with antimycin A in either darkness or photoinhibitory light. Other details were as in Figure 1.

slight decrease in photosynthesis of protoplasts in the presence of 1 μM antimycin A after preincubation, even under darkness, but the inhibition of photosynthesis was much more marked under illumination with photoinhibitory light (Fig. 3). When the activities were expressed in relation to their respective dark controls, pronounced photoinhibition in presence of antimycin A became very clear. After even 10 min of incubation under photoinhibitory light, the protoplasts retained only about 70% of photosynthetic activity compared to the dark control, whereas the activity dropped further to 35% in presence of antimycin A. The marginal loss of activity during incubation in darkness probably resulted because the stability of protoplasts was affected after 15 min at 30°C. All the subsequent experiments were therefore limited to 10 min of exposure/incubation.

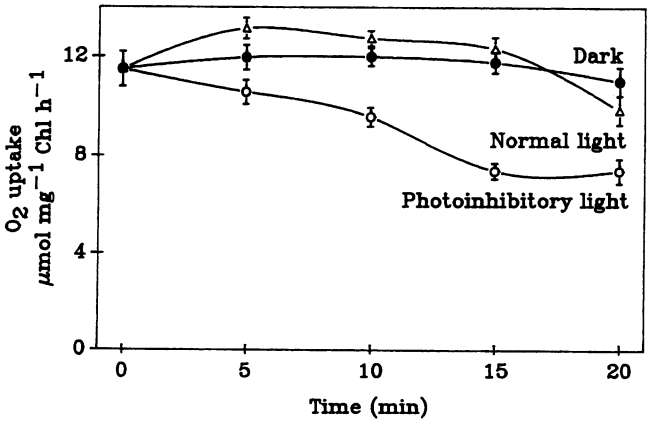

Figure 2. Inhibition of respiratory oxygen uptake in mesophyll protoplasts on exposure to photoinhibitory light, compared to the samples kept in darkness or normal light. Further details were as in Figure 1.

There was already a marked effect of antimycin A by 10 min (Fig. 3). The effective concentrations of three classic respiratory inhibitors were then determined in the next set of experiments by exposing the protoplasts to photoinhibitory light or darkness for 10 min. Photoinhibition was remarkably enhanced by the presence of even 1 μM antimycin A (Fig. 4A), 1 μM sodium azide (Fig. 4B), and 1 μg mL^{-1} of oligomycin (Fig. 4C).

These metabolic inhibitors could exert a direct inhibitory effect on photosynthesis. This aspect was checked by including the inhibitor in the assay medium while measuring photosynthesis. However, antimycin A, sodium azide, or oligomycin did not affect photosynthetic O_2 evolution by protoplasts at these low concentrations (data not shown, but see Table I for a similar experiment).

Antimycin A, sodium azide, and oligomycin inhibit oxidative electron transport and phosphorylation. The effect of two more inhibitors, sodium fluoride and sodium malonate, which interfere with glycolysis and the tricarboxylic acid cycle, respectively, were examined. However, sodium fluoride and sodium malonate did not have much effect on photoinhibition (Table I). On the other hand, antimycin A, sodium azide, or oligomycin markedly promoted photoinhibition of photosynthesis. At these concentrations and experimental incubatory conditions, the inhibition by test compounds of the respiratory activity was quite marked and ranged from 29 to 46% (Table II).

It is possible that these respiratory inhibitors (antimycin A, sodium azide, and oligomycin) may affect enzymes capable of scavenging H_2O_2, such as catalase or peroxidase. However, antimycin A or oligomycin did not have any effect on catalase or peroxidase when assayed in protoplasts, even at concentrations as high as 100 μM or 100 μg mL^{-1} (data not shown). Sodium azide did not inhibit peroxidase, but suppressed about 15 and 40% of catalase activity at concentrations of 1 and 10 μM, respectively.

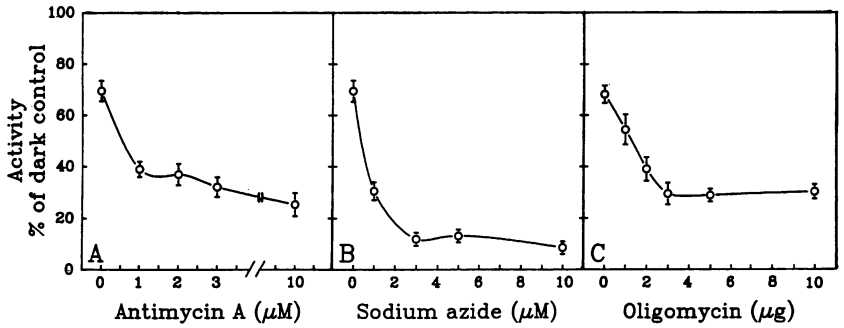

Figure 4. Further promotion of photoinhibition of photosynthesis in mesophyll protoplasts by classic respiratory inhibitors. Protoplasts were either kept in darkness or exposed to photoinhibitory light for 10 min at 30°C. The test inhibitors (A, antimycin A; B, sodium azide; C, oligomycin) were included at the indicated concentrations. The figures represent the activity after photoinhibition compared to the corresponding sample kept in darkness. The photosynthetic activity after photoinhibition in the absence of inhibitors (indicated at zero concentration in A, B, C) ranged from 65 to 74% of that in dark-incubated sample. Other details were as in Figure 1.

DISCUSSION

The present article establishes that the protoplasts can be employed to study the process of photoinhibition. There is a brief report on photoinhibition in protoplasts describing changes in fluorescence induction curves (3). Our observations further indicate that the oxidative metabolism in mitochondria plays an important role in protecting protoplasts against photoinhibition of photosynthesis.

Each experimental system, however, has its own advantages and disadvantages. For example, leaves have a nonuniform light profile between adaxial and abaxial surfaces and exhibit variation in partial pressures of CO_2/O_2 levels within intercellular spaces (14). The interaction between different organelles of a cell cannot be assessed while using chloroplasts. The system of protoplasts offers an additional tool to study the phenomenon of photoinhibition. An advantage is the possibility of assessing the interaction between organelles and testing the effect of exogenously added metabolites/inhibitors. The major disadvantage, however, is the limited stability of protoplasts, particularly at room temperature. Most of the experiments in the present report were limited to 10 min. Within this period, the loss in photosynthetic activity of pea mesophyll protoplasts was negligible (Fig. 1).

Plant systems have many defense mechanisms to minimize the damage of photoinhibition. Carbon dioxide and O_2, which are the basic substrates for photosynthesis and photorespiration, respectively, protect the plant cell against photoinhibition (11). The operation of PCR cycle facilitates a continuous supply of terminal electron acceptor of photochemical reactions (NADP) and permits a steady rate of photochemical deexcitation of reaction centers (11). In the absence of CO_2, the protection against photoinhibition is provided by photorespiratory carbon metabolism (9, 21).

Table I. *A Comparison of the Effect of Five Metabolic Inhibitors on Photosynthesis and Photoinhibition in Protoplasts*

These compounds affect different components of respiration—sodium fluoride (inhibits glycolysis), sodium malonate (tricarboxylic acid cycle), antimycin A, sodium azide (oxidative electron transport), and oligomycin (oxidative phosphorylation). The protoplasts were examined for their photosynthetic activity after a preincubation (with or without inhibitors) for 10 min at 30°C in either darkness or photoinhibitory light.

Respiratory Inhibitor	Photosynthetic Rate after Preincubation		Effect on Photosynthesis[a]	Photosynthesis after Photoinhibition
	Dark	Photoinhibitory light		
	μmol O_2 evolved mg^{-1} Chl h^{-1}		*% of control*	*% of respective dark treatment*
None (control)	122 ± 8	78 ± 5	100	64
10 mM Sodium fluoride	117 ± 5	70 ± 4	96	60
10 mM Sodium malonate	120 ± 5	69 ± 3	98	58
1 μM Antimycin A	100 ± 4	38 ± 5	82	38
1 μM Sodium azide	113 ± 6	38 ± 4	93	34
1 μg mL^{-1} Oligomycin	105 ± 4	48 ± 5	80	46

[a] Possible direct effect seen on incubation in darkness.

Table II. *Extent of Inhibition of Dark Respiration by Test Compounds under the Present Experimental Conditions*

The protoplasts were preincubated (with or without inhibitor) for 10 min at 30°C in darkness and were examined for their rate of respiratory oxygen uptake.

Metabolic Inhibitor	Respiratory Activity	
	Rate	Inhibition
	μmol O_2 uptake mg^{-1} Chl h^{-1}	% of control
None (control	8.4 ± 0.3	100
10 mM Sodium fluoride	5.9 ± 1.1	70
10 mM Sodium malonate	5.0 ± 1.3	60
1 μM Antimycin A	6.0 ± 0.5	71
1 μM Sodium azide	4.5 ± 1.3	54
1 μg mL^{-1} Oligomycin	5.1 ± 1.0	61

Some of the other mechanisms that alleviate the effects of photoinhibition include the violaxanthin cycle (*e.g.* see ref. 18) and the rapid responses in turnover of D1 protein (30).

The present report reveals that dark respiration forms an additional defense mechanism to protect the leaf cells against photoinhibition. Our suggestion is based on three observations: restriction of respiration by test compounds under present experimental conditions (Table II), decrease in respiratory rates due to photoinhibitory light (Fig. 2), and marked promotion of photoinhibition, even at very low concentrations of classic inhibitors of mitochondrial metabolism (Figs. 3 and 4; Table I).

The effect of O_2 on photoinhibition is intriguing. In an excess of light, O_2 forms superoxide radicals, which are harmful to the photosynthetic apparatus (5). Leaf cells are vulnerable to photooxidative damage because they are exposed to bright light while they produce dioxygen. The leaves are equipped to suppress the production and/or to remove immediately the superoxide radicals/H_2O_2. Superoxide dismutase and catalase are among the important factors that help the plant cells in scavenging of superoxide radicals, thus avoiding further photoinhibition (reviewed in ref. 2).

Some of these inhibitors could affect the scavenging of superoxide radicals by suppressing the activities of catalase or peroxidase to make the photosynthetic system more vulnerable. Antimycin A, sodium azide, or oligomycin do not affect catalase or peroxidase at the concentrations used during photoinhibitory treatment. Catalase is known to be inhibited by sodium azide (19), but at a much higher concentration than that required to promote photoinhibition (Fig. 4B; Table I). We do not know of any report on the inhibition of catalase/peroxidase by antimycin A or oligomycin. Antimycin also may affect photosynthetic carbon metabolism, but again at a high concentration and at certain conditions (25). Therefore, we suggest that the promotion of photoinhibition by low concentrations of antimycin A, sodium azide, or oligomycin is basically due to their interference with respiratory metabolism.

There are three possible factors that could facilitate respiration to protect the mesophyll protoplasts against photoinhibition. Respiratory metabolism could either (a) elevate the level of intracellular of CO_2, particularly at CO_2-limiting conditions, (b) provide extra energy toward the turnover of D1 protein required to prevent photoinhibition, or (c) help to maintain an optimal redox state in chloroplasts or cytosol of the cells. Sodium fluoride and malonate, which inhibit glycolysis and tricarboxylic acid cycle, respectively, would decrease the decarboxylation of carbon compounds but could not affect the extent of photoinhibition (Table I). Apparently, respiratory CO_2 evolution was not a major factor in modulating photoinhibition. Similarly, oligomycin, which inhibits oxidative phosphorylation, was not as effective as sodium azide or antimycin A (inhibitors of oxidative electron transport) in enhancing the extent of photoinhibition. Therefore, we believe that oxidative electron transport in mitochondria plays a much stronger role than the oxidative phosphorylation during protection of protoplasts against photoinhibition.

Active photosynthetic carbon metabolism in light promotes respiration during the subsequent dark period. This phenomenon of LEDR is already demonstrated in leaves and protoplasts (24, 26). The decrease in respiratory oxygen uptake after photoinhibitory treatment could, therefore, be a consequence of restriction in the photosynthetic capacity during the preceding light period.

Recent reports established that the oxidative metabolism in mitochondria has a beneficial and essential role in the photosynthetic process as well (12, 13, 28). It is possible that the marginal interference by respiratory inhibitors of photosynthetic metabolism (Table II) makes protoplasts highly susceptible to photoinhibitory light. Respiration has been proposed to prevent the over-reduction of the photosynthetic electron transport chain in chloroplasts by providing an outlet for reduced equivalents to the cytosol or mitochondria (12). We suggest that oxidative metabolism protects the plant cell against photoinhibition possibly by preventing the over-reduction of electron transport chain in chloroplasts.

CONCLUSIONS

Oxidative metabolism not only benefits photosynthesis, but also provides protection against photoinhibition in protoplasts. The system of protoplasts offers an additional tool to study the phenomenon of photoinhibition.

LITERATURE CITED

1. **Arnon DI** (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15
2. **Asada K, Takahashi M** (1987) Production and scavenging of active oxygen in photosynthesis. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Topics in Photosynthesis, Vol 9. Elsevier, Amsterdam, pp 227–287
3. **Baker NR, Horton P** (1987) Chlorophyll flourescence quenching during photoinhibition. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Topics in Photosynthesis, Vol 9. Elsevier, Amsterdam, pp 145–168
4. **Devi MT, Vani T, Reddy M, Raghavendra AS** (1992) Rapid isolation of mesophyll and guard cell protoplasts from the leaves of pea and maize. Indian J Exp Biol **30:** 424–428
5. **Egneus H, Heber U, Matthiesen U, Kirk M** (1975) Reduction of oxygen by the electron transport chain of chloroplasts during assimilation of carbon dioxide. Biochim Biophys Acta **408:** 252–268
6. **Graham D** (1980) Effects of light on dark respiration. *In* DD Davies, ed, Biochemistry of Plants, Vol 2. Academic Press, New York, pp 525–579

7. **Graham D, Chapman MD** (1979) Interactions between photosynthesis and respiration in higher plants. *In* M Gibbs, E Latzko, eds, The Encyclopaedia of Plant Physiology, New Series, Vol 6. Springer-Verlag, Berlin, pp 150–162
8. **Greer DH, Laing WA** (1989) Effect of temperature and PFD on the susceptibility of leaves to photoinhibition and recovery. *In* M Baltscheffsky, ed, Current Research in Photosynthesis, Vol II. Kluwer Academic, Dordrecht, pp 365–372
9. **Heber U, Schreiber U, Siebke K, Dietz KJ** (1990) Relationship between light-driven electron transport, carbon reduction and carbon oxidation in photosynthesis. *In* I Zelitch, ed, Perspectives in Biochemical and Genetic Regulation of Photosynthesis. Alan R. Liss Inc, New York, pp 1–37
10. **Krause GH** (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol Plant **74:** 566–574
11. **Krause GH, Cornic G** (1987) Carbon dioxide and oxygen interactions in photoinhibition. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Topics in Photosynthesis, Vol 9. Elsevier, Amsterdam, pp 169–195
12. **Krömer S, Heldt HW** (1991) On the role of mitochondrial oxidative phosphorylation in photosynthesis metabolism as studied by the effect of oligomycin on photosynthesis in protoplasts and leaves of barley (*Hordeum vulgare*). Plant Physiol **95:** 1270–1275
13. **Krömer S, Stitt M, Heldt HW** (1988) Mitochondrial oxidative phosphorylation participating in photosynthetic metabolism of a leaf cell. FEBS Lett **226:** 352–356
14. **Kyle DJ** (1987) The biochemical basis for photoinhibition of photosystem II. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Topics in Photosynthesis, Vol 9. Elsevier, Amsterdam, pp 197–226
15. **Kyle DJ, Ohad I** (1986) The mechanism of photoinhibition in higher plants and green algae. *In* LA Staehlin, CJ Arntzen, eds, Encyclopaedia of Plant Physiology, New Series, Vol 19. Springer-Verlag, Berlin, pp 468–474
16. **Kyle DJ, Ohad I, Arntzen CJ** (1984) Membrane protein damage and repair: selective loss of a quinone protein function in chloroplast membranes. Proc Natl Acad Sci USA **81:** 4070–4074
17. **Lidholm J, Gustafsson P, Öquist G** (1987) Photoinhibition of photosynthesis and its recovery in the green alga *Chlamydomonas reinhardii*. Plant Cell Physiol **28:** 1133–1140
18. **Moñtanes L, Heras L, Val J, Monge E** (1989) Zeaxanthin formation in etiolated leaves. *In* M Baltscheffsky, ed, Current Research in Photosynthesis, Vol II. Kluwer Academic, Dordrecht, pp 499–502
19. **Nakano Y, Asada K** (1980) Spinach chloroplasts scavenge hydrogen peroxide on illumination. Plant Cell Physiol **21:** 1295–1307
20. **Ohad I, Kyle DJ, Arntzen CJ** (1984) Membrane protein damage and repair: removal and replacement of inactivated 32 kD polypeptides in chloroplast membranes. J Cell Biol **99:** 481–485
21. **Osmond CB** (1981) Photorespiration and photoinhibition: some implications for the energetics of photosynthesis. Biochim Biophys Acta **639:** 72–98
22. **Powles SB** (1984) Photoinhibition of photosynthesis induced by visible light. Annu Rev Plant Physiol **35:** 15–44
23. **Powles SB, Björkman O** (1982) Photoinhibition of photosynthesis: Effect on chlorophyll fluorescence at 77K in intact leaves and in chloroplast membranes of *Nerium oleander*. Planta **156:** 97–107
24. **Reddy MM, Vani T, Raghavendra AS** (1991) Light-enhanced dark respiration in mesophyll protoplasts of pea. Plant Physiol **96:** 1368–1371
25. **Slovacek RE, Hind G** (1980) Energetic factors affecting carbon dioxide fixation in isolated chloroplasts. Plant Physiol **65:** 526–532
26. **Stokes D, Walker DA, Grof CPL, Seaton GGR** (1990) Light enhanced dark respiration. *In* I Zelitch, ed, Perspectives in Biochemical and Genetic Regulation of Photosynthesis, Alan R. Liss Inc, New York, pp 319–338
27. **Tolbert NE, Oeser A, Yamazaki RK, Hageman RH, Kisaki T** (1969) A survey of plants for leaf peroxisomes. Plant Physiol **44:** 135–147
28. **Vani T, Reddy MM, Raghavendra AS** (1990) Beneficial interaction between photosynthesis and respiration in mesophyll protoplasts of pea during short light-dark cycles. Physiol Plant **80:** 467–471
29. **Van Huyster RB, Loberzewski A** (1982) An immunological study of peroxide release by cultured peanut cells. Plant Sci Lett **27:** 59–67
30. **Wild A, Richter M, Rühle W** (1989) The mechanism of photoinhibition of spinach thylakoids. *In* M Baltscheffsky, ed, Current Research in Photosynthesis, Vol II. Kluwer Academic, Dordrecht, pp 377–380